

Chronic Sleep Disturbance Aggravates Peptic Ulcer in Rats through a Time-Ordered Vagal Cascade: Vagal Firing, Gastric Acid and Mucosal Inflammation, with the Vagus Nerve as the Initiating Step

Huang Du¹, Lianxiang Peng², Shuping Xiao², Rongpan Lin², Kangming Huang², Mingxia Wu², Nating Wei², Wangfeng Cai² and Hongbin Chen^{2*}

¹Department of Gastroenterology, Minqing County General Hospital, Fuzhou, Fujian Province, China

²Department of Gastroenterology, Sanming First Hospital Affiliated to Fujian Medical University, Sanming, China

***Corresponding Author:** Hongbin Chen, Department of Gastroenterology, Sanming First Hospital Affiliated to Fujian Medical University, Sanming, China.

Received: October 12, 2026; **Published:** October 26, 2026

Abstract

Objective: Chronic sleep disturbance aggravates peptic ulcer disease, yet the temporal ordering of vagal, secretory, inflammatory and mucosal events, and the role of the vagus nerve, remain unclear. We tested a time-ordered cascade (vagal hyperactivity to acid hypersecretion to mucosal inflammation to ulceration) and whether early versus late sleep restoration or subdiaphragmatic gastric vagotomy interrupts it.

Methods: 288 male Sprague-Dawley rats were allocated to six groups (control; ulcer alone; sleep deprivation with ulcer; early restoration, day 7; late restoration, day 21; vagotomy) and killed on days 3, 7, 14 or 28 (n = 12 per subgroup). Ulcers were induced by acetic acid injection; sleep deprivation used the modified multiple-platform method. Vagal firing, basal acid output, gastric pH and volume, IL-1 β , IL-6, IL-8/CINC-1, TNF- α mRNA, ulcer index and histology were measured serially; day-28 data were analyzed by animal-level serial-mediation SEM with Monte Carlo intervals.

Results: Vagal firing rose first, on day 3, when acid had not yet risen (P = 0.62); acid increased on day 7, cytokines on day 14, and ulceration peaked on day 28 (65.8 ± 5.6 vs 4.3 ± 3.8 mm² control; P < 0.001). Early restoration reduced the ulcer index to near ulcer-alone levels (15.0 ± 8.8), late restoration only partially (36.7 ± 4.3 ; P < 0.001), and vagotomy returned ulcers to ulcer-alone levels (11.6 ± 4.2 vs 12.8 ± 8.2 ; equivalence P = 0.019). The model fitted well (CFI = 0.996, RMSEA = 0.074) with a significant serial indirect path (β = 0.81; 95% CI 5.47-8.64) and non-significant direct paths, indicating full mediation.

Conclusion: Sleep disturbance aggravates peptic ulcer through a vagus-initiated, time-ordered acid-inflammation cascade, identifying the vagus as an upstream, time-sensitive therapeutic target.

Keywords: Sleep Deprivation; Peptic Ulcer; Vagus Nerve; Gastric Acid; Inflammatory Mediators; Rats

Introduction

Peptic ulcer disease still ranks among the leading digestive disorders worldwide; the 2019 Global Burden of Disease study placed its burden across 204 countries and territories [1]. Although falling *Helicobacter pylori* prevalence has reduced the incidence of uncomplicated ulcers, peptic ulceration remains a common cause of hospitalisation and of upper gastrointestinal bleeding [2,3]. Setting aside *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs, the imbalance between gastric acid-pepsin aggression and mucosal defence remains the central mechanism of ulcer formation and impaired healing [2,3]. Current management centres on acid suppression and *H. pylori* eradication; although effective for most patients, long-term proton-pump inhibitor use carries safety considerations, and a clinically important subset of ulcers remains refractory or recurrent, which has renewed interest in non-pharmacological and neuro-regulatory strategies directed at the vagus nerve [3].

Sleep disorders are highly prevalent: in a pooled analysis of 1.1 million adults, insomnia symptoms affected approximately 10%-20% of the population [5]. Psychosocial and sleep factors have drawn growing attention in digestive disorders in recent years, and sleep disturbance tracks closely with gastro-oesophageal reflux, functional gastrointestinal disease and mucosal injury [4].

Sleep is an important regulator of immune and inflammatory homeostasis: experimental and clinical evidence indicates that insufficient or fragmented sleep raises systemic pro-inflammatory cytokines and amplifies inflammatory responses [6-8]. Within the gastrointestinal tract, partial sleep deprivation compromises gastric mucosal integrity [9], and sleep loss aggravates oral ulcers and delays their healing [10]. More directly, total sleep deprivation delays gastric ulcer healing and impairs mucosal repair in rats [11], and genetic evidence supports a causal link between insomnia and an increased risk of peptic ulcer disease [12]. Yet most earlier studies examined single time points and single indicators, so the time-resolved neural pathway by which sleep disturbance translates into organic gastric mucosal damage remains poorly characterized.

The brain-gut axis offers a useful framework here. As the principal bidirectional pathway between brain and stomach, the vagus nerve controls gastric secretion and motility and forms the efferent arm of the cholinergic anti-inflammatory reflex [13-18]; vagal imbalance, in turn, has been linked to inflammation and gastrointestinal dysfunction [19]. At the gastric level, vagal-parietal cell signalling promotes basal and stimulated acid secretion through acetylcholine and histamine pathways [20], whereas acid exposure and *H. pylori* trigger epithelial release of IL-8 and other chemokines that recruit neutrophils, feeding an acid-inflammation positive feedback [21]. We therefore hypothesized a time-ordered cascade that, to our knowledge, has not been directly tested: sleep disturbance first heightens gastric vagal efferent firing, which then drives acid hypersecretion; sustained acid exposure amplifies mucosal inflammatory mediators, and the ulcer is aggravated and perpetuated.

Building on our previous work [22], we moved from a single-endpoint, five-group design to a time-resolved design with six groups and four sacrifice time points, and tested the hypothesis with three complementary strategies: (i) serial sampling, to identify the earliest time at which vagal firing, acid secretion, inflammation and ulceration deviate from control; (ii) two sleep-restoration windows (day 7 and day 21), to test the timing dependence of intervention; and (iii) bilateral subdiaphragmatic gastric vagotomy as an upstream ablation, together with animal-level serial-mediation SEM to separate direct from indirect pathways. Specifically, we asked whether the vagus nerve is the upstream initiating step of sleep-disturbance-aggravated ulceration, whether inflammation is the downstream amplifying step, and whether early intervention outperforms late intervention.

Materials and Methods

Animals and ethics

Two hundred eighty-eight healthy male SPF Sprague-Dawley rats (180-200g, 6-8 weeks old) were obtained from Guangdong Sijia Jingda Biotechnology Co. Ltd. After one week of acclimatization, the rats were housed in an SPF-grade environment at 22°C ± 2°C and 50%-60% relative humidity, under a 12-h light-dark cycle with free access to food and water. The study was carried out in strict accor-

dance with the Guide for the Care and Use of Laboratory Animals, approved by the Animal Ethics Committee of Fujian Medical University, and all procedures followed the ARRIVE 2.0 guidelines [23] and the 3Rs principles to minimize animal suffering.

Equipment and reagents

Key equipment included: a PowerLab multi-channel physiological recorder (ADInstruments) for vagal nerve electrical activity; a pocket centrifuge (Servicebio, D1008E); a decolorizing shaker (Servicebio, TSY-B); pipette guns (Dragon, KE0003087/KA0056573); an orthostatic fluorescence microscope (Nikon ECLIPSE C1, Japan); a developer (Tanon, CRB F119); and a fluorescence quantitative PCR instrument (BIO-RAD, CHROM04). Main reagents: acetic acid (Xilong Scientific Corporation); PBS buffer solution (Servicebio, G0002); RNA-easy fast animal tissue/cell total RNA extraction kit (TIANGEN, DP451-TA); universal tissue fixative (Servicebio, G1101-500ML); H&E high-definition staining kit (Servicebio, G1076); BeyoRT II cDNA first-strand synthesis kit (RNase H⁻, Beyotime, D7168M); BeyoFast SYBR Green qPCR Mix (2 $\times$, Low ROX, Beyotime, D7262-25 mL). Eszopiclone (2 mg/kg) was used for sleep restoration as in our previous protocol [22].

Groups, model establishment and sampling

Rats were randomly allocated to six groups: control (Ctrl), ulcer alone (Ulc), sleep deprivation with ulcer (SDU), early sleep restoration (Early; deprivation stopped and eszopiclone started on day 7), late sleep restoration (Late; deprivation stopped and eszopiclone started on day 21), and sleep deprivation with ulcer plus subdiaphragmatic gastric vagotomy (VagX). Each group was randomly sampled at four time points (days 3, 7, 14 and 28 after ulcer induction), with 12 rats per subgroup, for a total of 288. The acetic acid ulcer model was created by injecting 0.05 mL of 20% acetic acid into the gastric wall [24,25], consistent with our previous system [22]; control rats received sham surgery only. At each time point, rats were euthanized after recordings and blood collection, and gastric juice, serum and gastric tissue were harvested.

Chronic sleep deprivation

Chronic paradoxical (REM) sleep deprivation was induced with the modified multiple-platform method (MMPM) [26]. Rats were kept on narrow platforms above water; the muscle atonia that accompanies REM sleep made them touch the water and wake up. Deprivation started on the day of ulcer induction and continued according to each group's schedule. To limit water- and stress-related confounding, cage adaptation and large-platform controls were used. In the Early and Late groups, normal sleep was restored from days 7 and 21, respectively, and eszopiclone (2 mg/kg) was administered until day 28.

Subdiaphragmatic gastric vagotomy

In the VagX group, bilateral subdiaphragmatic gastric vagal trunks were transected at the time of ulcer induction, removing gastric vagal efferent and afferent innervation; the other groups underwent identical exposure without nerve transection (sham surgery). Denervation was confirmed functionally by blunted gastric distension responses and loss of centrally stimulated acid secretion [22].

Gastric vagal nerve electrical activity

Under pentobarbital anesthesia, a gastric vagal branch was isolated and placed on a double-hook silver wire recording electrode, and spontaneous discharge was recorded with the PowerLab multi-channel physiological recorder. Firing frequency (imp/s) and amplitude difference (μ V) were measured from a stable segment, under blinded conditions, across groups.

Gastric acid and juice measurements

Gastric juice was collected for 1h by pyloric ligation; volume (mL) and pH were recorded, and basal acid output (mmol H⁺/h) was determined by acid-base titration.

Inflammatory mediator mRNA measurement

Total RNA was extracted from gastric mucosa and serum with the RNA-easy kit, and cDNA was synthesized with BeyoRT II. qPCR for IL-1 β , IL-6, IL-8 and TNF- α was run with BeyoFast SYBR Green Mix on the BIO-RAD CHROMO4 instrument. Because rats lack a direct IL-8 ortholog, the functional homolog CINC-1/CXCL1 system was measured and uniformly reported as IL-8 (CINC-1/CXCL1). GAPDH served as the internal reference, and relative expression was calculated with the 2^{- $\Delta\Delta$ Ct} method [22].

Ulcer index and histopathology

Ulcer area was measured macroscopically and expressed as the ulcer index (mm²). Gastric tissues were fixed, embedded, sectioned and stained with H&E, after which two blinded pathologists scored inflammatory infiltration, necrosis and granulation tissue on a 0-4 semi-quantitative scale.

Statistical analysis

Each rat was treated as the independent experimental unit (n = 12 per subgroup), and data are expressed as mean $\pm$ SD. Normality was assessed with the Shapiro-Wilk test. Between-group comparisons at each time point used one-way ANOVA with Tukey HSD post hoc tests, group $\times$ time effects were tested by two-way ANOVA (Type III sums of squares with sum coding), and non-normal units were verified with the Kruskal-Wallis H test. On day 28, a latent-variable serial-mediation SEM was fitted at the animal level across the five continuous groups (Ctrl, Ulc, SDU, Early, Late; n = 60). The vagal latent variable V was indicated by firing frequency and amplitude, the inflammatory latent variable I by the four cytokines, and the structure was acid $\leftarrow$ V, inflammation $\leftarrow$ acid, ulcer $\leftarrow$ inflammation + V + acid. Parametric Monte Carlo intervals (B = 20,000) based on ML estimates and asymptotic standard errors served as the primary inference for the serial indirect effect, with stratified non-parametric bootstrap (B = 1,000) as sensitivity analysis. VagX was analyzed separately as surgical ablation using Welch’s t test and two one-sided equivalence testing (TOST) against the ulcer-alone group, with a pre-specified bound of $\pm$ 7 mm². Two-sided P < 0.05 was considered significant.

Results

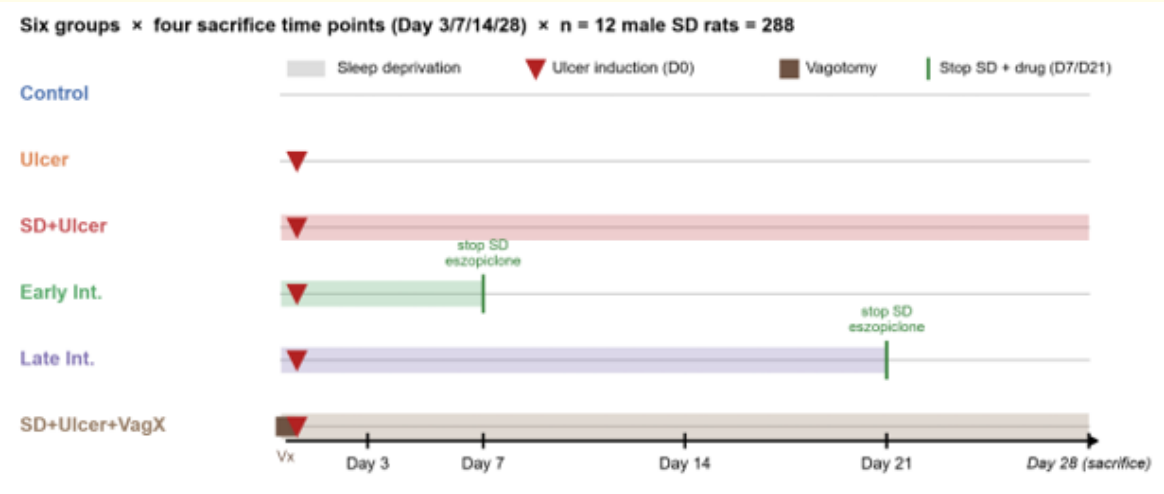


Figure 1: Experimental design and group timeline (six groups $\times$ four sacrifice time points $\times$ n = 12 = 288).

Vagal firing, acid, inflammation and ulceration deviate in a time-ordered sequence

Differences between the SDU and control groups emerged in a fixed order: nerve, secretion, inflammation, then damage. Gastric vagal firing frequency and amplitude rose as early as day 3 (frequency 24.2 vs 12.0 imp/s, Tukey $P < 0.001$), at a time when basal acid output, gastric pH and volume had not yet changed (acid $P = 0.62$). Acid-related parameters increased only on day 7 and stayed elevated (all $P < 0.001$). The four cytokines were indistinguishable from control on days 3 and 7 (IL-1 β day 7 $P = 0.94$) but rose together on day 14 (IL-1 β 3.25, ~3.3-fold control; IL-6, IL-8 and TNF- α similarly upregulated; all $P < 0.001$), climbing further by day 28 (IL-1 β 4.56, ~4.3-fold control). In the SDU group, the ulcer index rose progressively, from about 31 mm² on day 3 to 65.80 mm² on day 28. Two-way ANOVA detected significant group $\times$ time interactions for all indicators (all $P < 0.001$; Figure 2).

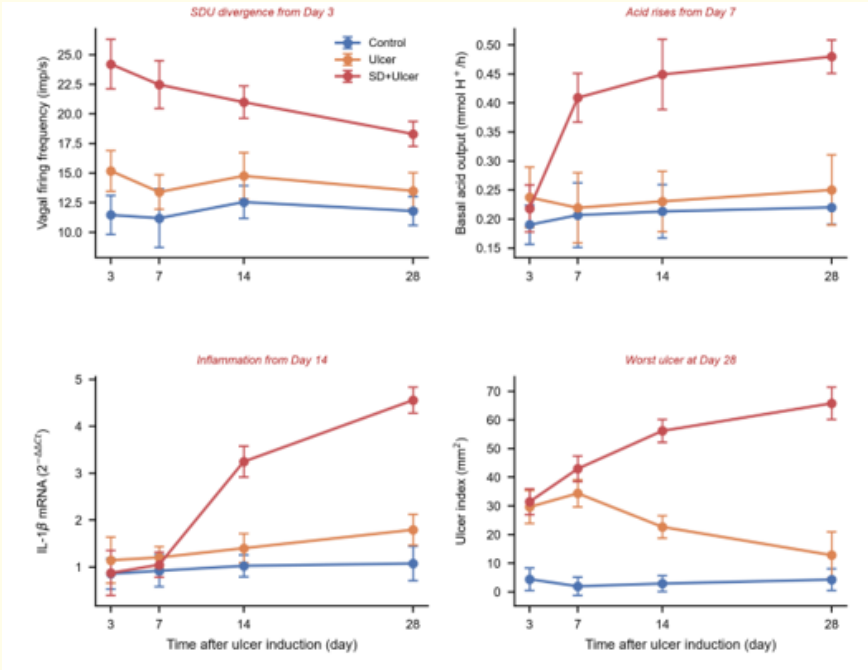


Figure 2: Temporal cascade: vagal firing (Day 3), gastric acid (Day 7), inflammation (Day 14) and ulceration (Day 28).

Day-28 endpoints: Sleep deprivation aggravates ulcers; early restoration is near-complete, late only partial

At day 28, every indicator differed significantly across the six groups. The SDU group reached an ulcer index of 65.80 ± 5.63 mm², well above control (4.27 ± 3.78 mm², $P = 5.3 \times 10^{-18}$) and ulcer-alone (12.80 ± 8.17 mm²). The Early group (15.00 ± 8.82 mm²) remained significantly above control (4.27 ± 3.78 mm², $P = 0.0008$), returned close to ulcer-alone levels (12.80 ± 8.17 mm², $P = 0.95$), and was significantly lower than the Late group (36.70 ± 4.27 mm², $P = 9.9 \times 10^{-7}$). The accompanying indicators followed the same gradient: vagal firing, acid and all four cytokines were highest in SDU, lowest in Early (near Ulc) and intermediate in Late (Table 1 and figure 3).

Subdiaphragmatic gastric vagotomy abolishes the cascade and returns ulcers to ulcer-alone levels

In the VagX group, gastric vagal firing was effectively eliminated (day-28 frequency 2.57 ± 1.85 imp/s), acid output fell to 0.08 ± 0.03 mmol H⁺/h, and the ulcer index was 11.63 ± 4.15 mm², significantly lower than in SDU ($P = 2.7 \times 10^{-17}$) and statistically indistinguishable

Indicator	Ctrl	Ulc	SDU	Early	Late	VagX
Vagal firing frequency (imp/s)	11.80 ± 1.22	13.50 ± 1.54	18.30 ± 1.05	12.50 ± 1.57	15.60 ± 1.93	2.57 ± 1.85
Basal acid output (mmol H ⁺ /h)	0.22 ± 0.03	0.25 ± 0.06	0.48 ± 0.03	0.23 ± 0.04	0.37 ± 0.05	0.08 ± 0.03
IL-1β mRNA	1.07 ± 0.37	1.79 ± 0.33	4.56 ± 0.28	1.58 ± 0.31	3.06 ± 0.37	1.83 ± 0.40
IL-6 mRNA	1.01 ± 0.33	1.56 ± 0.35	4.02 ± 0.28	1.44 ± 0.34	2.64 ± 0.34	1.58 ± 0.23
IL-8/CINC-1 mRNA	1.00 ± 0.38	1.89 ± 0.42	4.13 ± 0.26	1.55 ± 0.40	2.82 ± 0.32	1.82 ± 0.35
TNF-α mRNA	0.94 ± 0.25	1.51 ± 0.32	3.61 ± 0.21	1.46 ± 0.32	2.41 ± 0.32	1.43 ± 0.35
Ulcer index (mm ²)	4.27 ± 3.78	12.80 ± 8.17	65.80 ± 5.63	15.00 ± 8.82	36.70 ± 4.27	11.63 ± 4.15

Table 1: Day-28 outcomes across groups (mean ± SD, n = 12).

mRNA: Relative Expression by 2^{-ΔΔCt} (GAPDH). Early, deprivation stopped on day 7 + eszopiclone; Late, day 21; VagX, vagotomy.

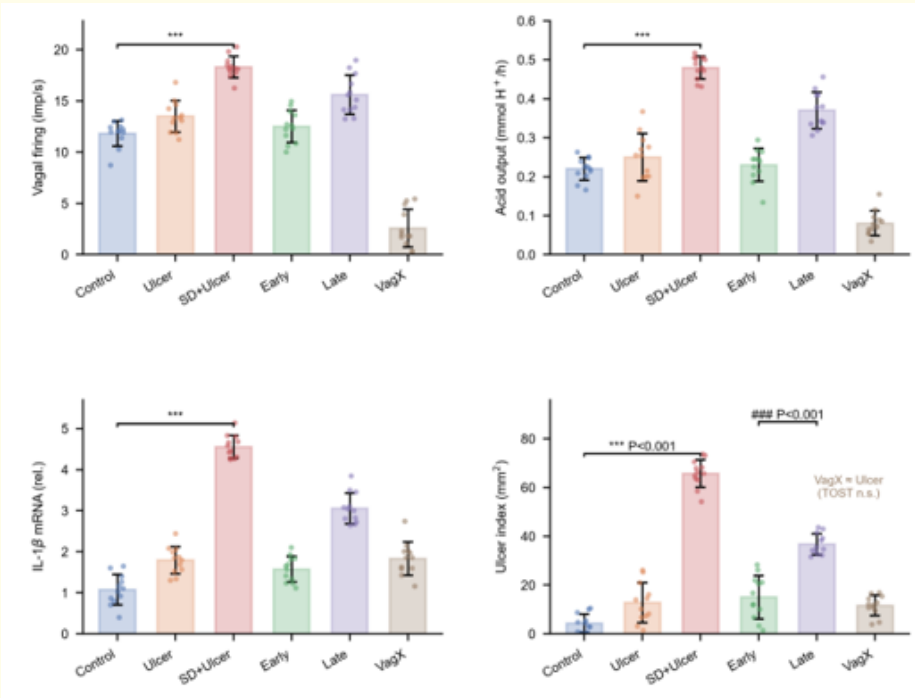


Figure 3: Day-28 comparison of vagal firing, gastric acid, IL-1β and ulcer index across six groups (***) vs control; ### Early vs Late).

from ulcer-alone ($12.80 \pm 8.17 \text{ mm}^2$; Welch $P = 0.66$). TOST confirmed equivalence within the pre-specified $\pm 7 \text{ mm}^2$ bound ($P = 0.019$). Removing gastric vagal innervation thus returned the injury to the level of ulcer-alone under sleep deprivation, identifying the vagus as a necessary upstream component of the aggravation.

Animal-level serial mediation: inflammation fully mediates; direct vagal and acid pathways are not significant

The animal-level latent-variable SEM on day 28 (five continuous groups, $n = 60$) showed excellent fit ($\chi^2(17) = 22.46$, $P = 0.17$; CFI = 0.996, TLI = 0.993, RMSEA = 0.074). Standardized loadings were 0.97 for both vagal indicators and 0.99 for all four inflammatory indicators. Structurally, vagus→acid ($\beta = 0.95$), acid→inflammation ($\beta = 0.96$) and inflammation→ulcer ($\beta = 0.89$) were each significant (all $P < 0.001$), whereas the direct vagus→ulcer path ($\beta = -0.10$, $P = 0.26$) and the direct acid→ulcer path ($\beta = 0.19$, $P = 0.10$) were not. The serial indirect effect (vagus→acid→inflammation→ulcer) was 6.96, with a parametric Monte Carlo 95% CI of 5.47-8.64 (standardized $\beta = 0.81$) that excluded zero; the stratified non-parametric bootstrap was directionally consistent. Along with the non-significant direct paths, these results support full serial mediation by inflammation (Figure 4). Because the SEM uses cross-sectional endpoint data, it reflects a path structure consistent with the causal cascade rather than a unique proof of causal direction (See discussion).

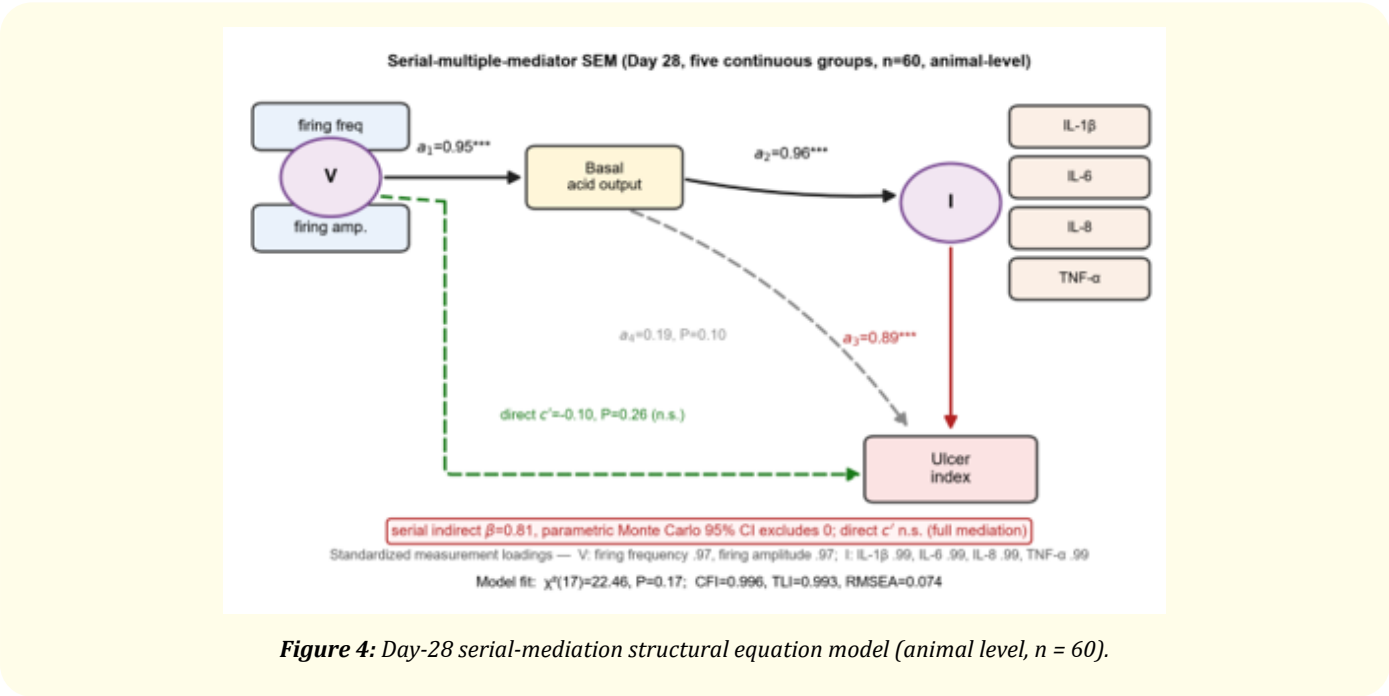


Figure 4: Day-28 serial-mediation structural equation model (animal level, $n = 60$).

Histopathology and non-parametric verification

Day-28 H&E semi-quantitative scores (0-4) ran parallel to the ulcer-index gradient (Table S1 and figure S1). Inflammatory infiltration and necrosis were highest in the SDU group (3.71 ± 0.26 and 3.62 ± 0.38), significantly greater than in every other group (Mann-Whitney U with Bonferroni correction, adjusted $P < 0.001$), which also had the lowest granulation tissue score (0.58 ± 0.42 ; adjusted $P < 0.001$ versus all groups). Early restoration yielded near-control scores (inflammation 0.96 ± 0.58 ; necrosis 0.71 ± 0.54), while Late restoration fell in between (2.25 ± 0.26 and 2.04 ± 0.40). Relative to ulcer-alone, vagotomy did not further reduce inflammation or necrosis (0.75 ± 0.50 vs 0.83 ± 0.62 , and 0.54 ± 0.50 vs 0.62 ± 0.53 ; adjusted $P > 0.05$) but did increase granulation tissue (2.79 ± 0.62 vs 2.00 ± 0.52 ; adjusted $P = 0.033$). Kruskal-Wallis tests were significant for all three scores (all $P < 0.001$). Of the 240 measurement units, 13 of 239 with complete

data (5.4%) deviated from normality on the Shapiro-Wilk test (Table S2), and the Kruskal-Wallis results agreed with the parametric analyses at all time points, including the non-significant differences for IL-1 β , IL-6, IL-8 and TNF- α on days 3 and 7.

Discussion

With a time-resolved design, this study assembles an internally consistent mechanistic chain for how sleep disturbance aggravates peptic ulceration: vagal firing hyperactivity (day 3) came first, acid hypersecretion followed (day 7), the mucosal inflammatory burst appeared on day 14, and ulceration progressed to a peak on day 28. Early sleep restoration was near-completely protective, late restoration only partially so, and gastric vagotomy abolished the cascade. Animal-level SEM indicated that the effects of vagus and acid on ulceration were mediated almost entirely by the inflammatory latent variable, with direct paths non-significant. Taken together, the timing, time-window, ablation and mediation evidence support a working model in which the vagus nerve is the upstream initiating step and inflammation the downstream amplifying step.

Mechanistically, sleep deprivation can raise vagal drive and tilt sympathetic/parasympathetic balance through hypothalamic-brain-stem autonomic networks [6,8,19]; enhanced vagal outflow, in turn, promotes acid secretion via parietal-cell and enterochromaffin-like pathways [20]. Sustained acid exposure damages the mucosal barrier and induces epithelial release of IL-8/CINC-1 chemokines that recruit neutrophils and activated macrophages [21]; IL-1 β , IL-6 and TNF- α are subsequently amplified, forming an acid-inflammation positive feedback that suppresses repair and aggravates ulceration. It is worth noting that the classical cholinergic anti-inflammatory reflex emphasizes vagal inhibition of cytokines through $\alpha 7$ nicotinic receptors [15-18]. The gastric pro-secretory, pro-inflammatory direction observed here suggests the net vagal effect is organ- and pathway-specific: in the stomach, chronically enhanced vagal drive may promote secretion first and override the systemic anti-inflammatory action in both magnitude and time. This organ-specific, time-dependent interpretation may reconcile seemingly conflicting literature, although selective afferent/efferent blockade and $\alpha 7$ antagonism experiments are needed to settle it.

Our findings also extend and refine earlier work on sleep and gastric damage. Guo., *et al.* found that 7 - 14 days of partial sleep deprivation compromised gastric mucosal integrity in roughly 30%-50% of rats, with increased gastric acidity and elevated plasma noradrenaline, gastrin and histamine alongside reduced mucosal blood flow [9]. Chen., *et al.* reported that sleep deprivation worsened oral ulcers and delayed healing in rats [10], and Anifowose., *et al.* recently showed that sleep deprivation impairs gastric ulcer healing and induces anxiety-like behaviour [11]. These studies are directionally consistent with ours and collectively indicate that sleep loss shifts gastric autonomic, secretory and inflammatory homeostasis toward mucosal injury. However, they evaluated single time points and single indicators, without vagal electrophysiology, serial sampling or intervention windows. By contrast, our time-resolved design shows that vagal hyperactivity precedes acid hypersecretion and mucosal inflammation, and that vagotomy eliminates the aggravation, placing the vagus upstream of the secretory and inflammatory changes reported previously.

These results are also relevant to the emerging field of vagal neuromodulation. The vagus is an approved therapeutic target in drug-resistant epilepsy and depression, and clinical evidence now supports its anti-inflammatory action: in patients with rheumatoid arthritis, implantable vagus nerve stimulation inhibited peripheral blood production of TNF, IL-1 β and IL-6 and improved disease activity [27], and in Crohn's disease, chronic vagus nerve stimulation induced clinical, biological and endoscopic remission with restored vagal tone in a pilot study [28]. Bioelectronic approaches are being developed across chronic inflammatory diseases [18]. Our data refine this picture for the stomach. Because chronically enhanced vagal drive promoted secretion first and amplified inflammation downstream, vagal modulation in peptic ulcer disease may need to reduce excessive gastric vagal outflow at an early stage, rather than globally enhancing cholinergic anti-inflammatory tone, and non-invasive devices that can be applied early, before the inflammatory amplifier is engaged, warrant investigation. The time-dependence we observed suggests that the success of such neuromodulatory strategies may hinge on when they are applied relative to the onset of mucosal inflammation.

The time-window results carry fairly direct translational implications. Restoring sleep at day 7, before the inflammatory burst, compressed the endpoint ulcer to near ulcer-alone levels; intervening at day 21, after the cascade had been established, was only partially effective. Once the inflammatory positive feedback has formed, simply removing the upstream stimulus cannot fully reverse the damage, a pattern consistent with inflammatory memory and delayed tissue injury [6,7,10]. For ulcer patients with severe sleep disturbance, early sleep assessment and intervention may therefore offer benefits beyond symptomatic management. In practice, this argues for screening sleep complaints in patients with recurrent or refractory ulcers, and for considering sleep restoration—sleep hygiene, cognitive behavioural therapy for insomnia or judicious hypnotic use—early in the disease course, before mucosal inflammation becomes self-sustaining. It also implies that the therapeutic window for vagal or cholinergic interventions may be wider when applied before the downstream inflammatory amplifier is fully engaged.

Limitation of the Study

This study has limitations. First, the SEM rests on day-28 cross-sectional data; although directionally consistent with the prospective timing and ablation evidence, mediation alone cannot establish causal direction. Longitudinal mediation, continuous *in vivo* vagal telemetry and selective fibre transection would be needed. Second, the modified multiple-platform method brings water exposure, movement restriction and HPA-axis stress; despite large-platform/environmental controls, stress cannot be fully excluded as a shared upstream factor, and corticosterone monitoring and non-aquatic sleep manipulation (e.g. gentle handling) should be added. Third, bilateral subdiaphragmatic transection cannot separate afferent from efferent contributions and may itself affect gastric emptying and nutrition. Fourth, only male rats, a single acetic-acid model and a single age range were used, which limits extrapolation to females, other ulcer types and humans. Fifth, eszopiclone may itself affect autonomic tone and inflammation; the design combines sleep restoration with drug administration, so a drug-only control is needed to isolate the drug's independent effect. Sixth, IL-8 in rats is represented by CINC-1/CXCL1, and cross-species comparisons should account for nomenclature and receptor differences.

Conclusion

Chronic sleep disturbance aggravates rat peptic ulceration through a time-ordered, vagus-initiated cascade of gastric acid hypersecretion and mucosal inflammation; the vagus nerve appears to be an upstream, intervenable initiating step, and inflammation a fully mediating downstream amplifier. Early sleep restoration was near-complete in its effect, late restoration only partial, and gastric vagotomy abolished the cascade. These findings offer mechanistic and time-window evidence for sleep management and vagal/cholinergic-targeted strategies in ulcer prevention and treatment, pending validation by longitudinal, selectively denervated and multi-model studies.

Author Contributions

Huang Du and Hongbin Chen designed the experiment. Rongpan Lin and Kangming Huang performed vagotomy on rats. Shuping Xiao constructed the rat ulcer markers and vagal nerve electrode implantation. Wangfeng Cai built a rat model of chronic sleep deprivation. Lianxiang Peng measured gastric vagal electrical activity. Huang Du recorded gastric acid secretion and ulcer healing in rats. Mingxia Wu and Nating Wei organized the data. Huang Du drafted the manuscript. Hongbin Chen reviewed and revised the manuscript.

Funding Support

Natural Science Foundation of Fujian Province (2024J011491); Sanming Science and Technology Program (2022-S-65).

Acknowledgments

We thank our colleagues and technicians for their great help and cooperation during the study.

Ethics Statement

The study was conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals and was approved by the Animal Ethics Committee of Fujian Medical University. All procedures were designed to ensure animal welfare and minimize suffering, in line with the 3Rs principles.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting Information

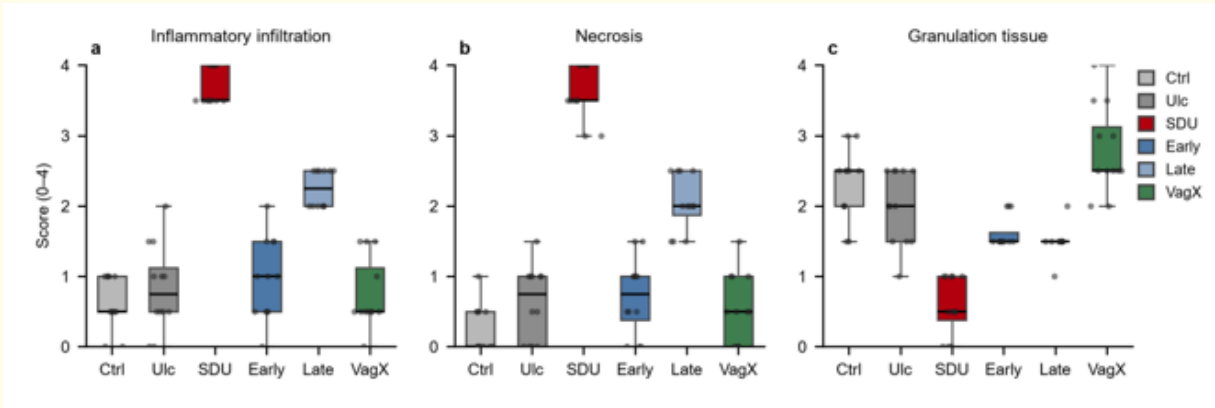


Figure S1: Day-28 histology semi-quantitative scores (0-4) by group.

Boxes show the interquartile range, whiskers $1.5 \times IQR$, and individual rats ($n = 12$ per group) are overlaid as points. (a) Inflammatory infiltration; (b) necrosis; (c) granulation tissue. Kruskal-Wallis tests across the six groups: $P = 8.7 \times 10^{-10}$ (a), $P = 4.5 \times 10^{-10}$ (b), $P = 3.5 \times 10^{-10}$ (c).

Score (0-4)	Ctrl (n = 12)	Ulc (n = 12)	SDU (n = 12)	Early (n = 12)	Late (n = 12)	VagX (n = 12)	Kruskal-Wallis P
Inflammatory infiltration	0.62 ± 0.38	0.83 ± 0.62	3.71 ± 0.26	0.96 ± 0.58	2.25 ± 0.26	0.75 ± 0.50	8.72×10 ⁻¹⁰
Necrosis	0.25 ± 0.34	0.62 ± 0.53	3.62 ± 0.38	0.71 ± 0.54	2.04 ± 0.40	0.54 ± 0.50	4.48×10 ⁻¹⁰
Granulation tissue	2.29 ± 0.50	2.00 ± 0.52	0.58 ± 0.42	1.62 ± 0.23	1.50 ± 0.21	2.79 ± 0.62	3.54×10 ⁻¹⁰

Table S1: Day-28 histology semi-quantitative scores by group (mean ± SD, n = 12).

Values are mean ± SD; n = 12 per group. Kruskal-Wallis H test across the six groups. Pairwise comparisons reported in the text used the Mann-Whitney U test with Bonferroni correction for six comparisons.

Variable	Group	Day	W	P
Vagal firing frequency	Ctrl	D28	0.826	0.0190
Vagal firing frequency	VagX	D3	0.809	0.0120
Vagal firing frequency	VagX	D7	0.687	0.0006
Vagal firing frequency	VagX	D14	0.860	0.0489
Vagal amplitude difference	VagX	D3	0.841	0.0287
Gastric juice volume	VagX	D14	0.847	0.0338
Gastric pH	SDU	D7	0.729	0.0016
Gastric pH	Late	D28	0.477	0.0000
IL-1 β mRNA	Ctrl	D7	0.837	0.0257
IL-1 β mRNA	Late	D7	0.850	0.0372
IL-8/CINC-1 mRNA	SDU	D14	0.788	0.0068
Ulcer index	Ctrl	D7	0.595	0.0001
Ulcer index	Ulc	D14	0.797	0.0087

(A) Shapiro-Wilk deviations from normality (13 of 239 units with complete data, 5.4%).

Indicator	Day	ANOVA F	ANOVA P	KW H	KW P
Vagal firing frequency (imp/s)	D3	297.39	7.72×10^{-44}	62.31	4.04×10^{-12}
Vagal firing frequency (imp/s)	D7	252.07	1.39×10^{-41}	59.97	1.24×10^{-11}
Vagal firing frequency (imp/s)	D14	126.73	1.90×10^{-32}	59.19	1.78×10^{-11}
Vagal firing frequency (imp/s)	D28	141.88	6.50×10^{-34}	57.84	3.40×10^{-11}
Vagal amplitude difference (μ V)	D3	258.01	6.71×10^{-42}	61.83	5.08×10^{-12}
Vagal amplitude difference (μ V)	D7	182.98	2.85×10^{-37}	59.70	1.40×10^{-11}
Vagal amplitude difference (μ V)	D14	112.53	6.40×10^{-31}	59.15	1.82×10^{-11}
Vagal amplitude difference (μ V)	D28	233.12	1.60×10^{-40}	59.93	1.26×10^{-11}
Basal acid output (mmol H ⁺ /h)	D3	17.62	4.72×10^{-11}	33.40	3.14×10^{-06}
Basal acid output (mmol H ⁺ /h)	D7	73.71	1.16×10^{-25}	57.71	3.61×10^{-11}
Basal acid output (mmol H ⁺ /h)	D14	80.00	1.18×10^{-26}	59.33	1.67×10^{-11}
Basal acid output (mmol H ⁺ /h)	D28	131.17	6.83×10^{-33}	60.50	9.58×10^{-12}
Gastric juice volume (mL)	D3	10.52	1.87×10^{-07}	26.77	6.32×10^{-05}
Gastric juice volume (mL)	D7	59.67	3.69×10^{-23}	54.81	1.43×10^{-10}
Gastric juice volume (mL)	D14	69.54	5.79×10^{-25}	59.01	1.95×10^{-11}
Gastric juice volume (mL)	D28	126.51	2.00×10^{-32}	59.52	1.53×10^{-11}
Gastric pH	D3	57.00	1.25×10^{-22}	32.83	4.06×10^{-06}
Gastric pH	D7	119.17	1.18×10^{-31}	58.78	2.17×10^{-11}
Gastric pH	D14	106.58	3.14×10^{-30}	59.96	1.24×10^{-11}
Gastric pH	D28	51.24	2.04×10^{-21}	55.25	1.16×10^{-10}
IL-1 β mRNA	D3	0.79	5.59×10^{-01}	2.66	7.52×10^{-01}
IL-1 β mRNA	D7	1.07	3.82×10^{-01}	5.49	3.59×10^{-01}
IL-1 β mRNA	D14	137.66	1.61×10^{-33}	56.92	5.26×10^{-11}

IL-1 β mRNA	D28	162.63	1.05×10^{-35}	57.77	3.51×10^{-11}
IL-6 mRNA	D3	0.98	4.37×10^{-01}	4.68	4.56×10^{-01}
IL-6 mRNA	D7	2.05	8.31×10^{-02}	10.19	6.99×10^{-02}
IL-6 mRNA	D14	62.73	9.64×10^{-24}	51.47	6.94×10^{-10}
IL-6 mRNA	D28	149.13	1.45×10^{-34}	56.08	7.82×10^{-11}
IL-8/CINC-1 mRNA	D3	0.69	6.29×10^{-01}	6.01	3.05×10^{-01}
IL-8/CINC-1 mRNA	D7	1.18	3.28×10^{-01}	5.32	3.78×10^{-01}
IL-8/CINC-1 mRNA	D14	40.75	6.70×10^{-19}	46.66	6.68×10^{-09}
IL-8/CINC-1 mRNA	D28	115.34	3.09×10^{-31}	57.97	3.20×10^{-11}
TNF- α mRNA	D3	1.12	3.59×10^{-01}	4.38	4.96×10^{-01}
TNF- α mRNA	D7	1.26	2.93×10^{-01}	7.01	2.20×10^{-01}
TNF- α mRNA	D14	66.92	1.66×10^{-24}	50.90	9.09×10^{-10}
TNF- α mRNA	D28	125.33	2.65×10^{-32}	56.42	6.66×10^{-11}
Ulcer index (mm ²)	D3	57.74	8.87×10^{-23}	46.91	5.93×10^{-09}
Ulcer index (mm ²)	D7	196.19	3.35×10^{-38}	56.47	6.50×10^{-11}
Ulcer index (mm ²)	D14	346.70	6.02×10^{-46}	65.91	7.24×10^{-13}
Ulcer index (mm ²)	D28	169.37	3.04×10^{-36}	56.03	8.01×10^{-11}

(B) Kruskal-Wallis versus one-way ANOVA across all measurement units ($n = 40$).

Table S2: Normality verification and agreement between non-parametric and parametric tests.

P values are shown as reported by SciPy. “Consistent” indicates that the Kruskal-Wallis and ANOVA conclusions ($P < 0.05$ or not) agree at that time point.

Bibliography

1. Ren J., *et al.* “The global burden of peptic ulcer disease in 204 countries and territories from 1990 to 2019: a systematic analysis for the Global Burden of Disease Study 2019”. *International Journal of Epidemiology* 51.5 (2022): 1666-1676.
2. Tuerk E., *et al.* “Peptic ulcer disease”. *Primary Care* 50.3 (2023): 351-362.
3. Lanas A, Chan FKL. “Peptic ulcer disease”. *The Lancet* 390.10094 (2017): 613-624.
4. Orr WC., *et al.* “The effect of sleep on gastrointestinal functioning in common digestive diseases”. *The Lancet Gastroenterology and Hepatology* 5.6 (2020): 616-624.
5. Kocavska D., *et al.* “Sleep characteristics across the lifespan in 1.1 million people from the Netherlands, United Kingdom and United States: a systematic review and meta-analysis”. *Nature Human Behaviour* 5.1 (2021): 113-122.
6. Irwin MR. “Sleep and inflammation: partners in sickness and in health”. *Nature Reviews Immunology* 19.11 (2019): 702-715.
7. Irwin MR., *et al.* “Sleep disturbance, sleep duration, and inflammation: a systematic review and meta-analysis of cohort studies and experimental sleep deprivation”. *Biological Psychiatry* 80.1 (2016): 40-52.

8. Greenlund IM, Carter JR. "Sympathetic neural responses to sleep disorders and insufficiencies". *American Journal of Physiology-Heart and Circulatory Physiology* 322.3 (2022): H337-H349.
9. Guo JS, et al. "Partial sleep deprivation compromises gastric mucosal integrity in rats". *Life Sciences* 77.2 (2005): 220-229.
10. Chen P, et al. "Sleep deprivation worsened oral ulcers and delayed healing process in an experimental rat model". *Life Sciences* 232 (2019): 116594.
11. Anifowose OF, et al. "Sleep deprivation impairs gastric ulcer healing and induces anxiety-like behavior in rats". *BMC Research Notes* 19.1 (2026): 360.
12. Zha LF, et al. "Effects of insomnia on peptic ulcer disease using Mendelian randomization". *Oxidative Medicine and Cellular Longevity* (2021): 2216314.
13. Cryan JF, et al. "The microbiota-gut-brain axis". *Physiological Reviews* 99.4 (2019): 1877-2013.
14. Bonaz B, et al. "The vagus nerve at the interface of the microbiota-gut-brain axis". *Cold Spring Harbor Perspectives in Medicine* 9.2 (2019): a034199.
15. Borovikova LV, et al. "Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin". *Nature* 405.6785 (2000): 458-462.
16. Tracey KJ. "The inflammatory reflex". *Nature* 420.6917 (2002): 853-859.
17. Rosas-Ballina M, et al. "Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit". *Science* 334.6052 (2011): 98-101.
18. Eberhardson M, et al. "Neural control of inflammation: bioelectronic medicine in treatment of chronic inflammatory disease". *Cold Spring Harbor Perspectives in Medicine* 10.3 (2020): a034181.
19. Breit S, et al. "Vagus nerve as modulator of the brain-gut axis in psychiatric and inflammatory disorders". *Frontiers in Psychiatry* 9 (2018): 44.
20. Schubert ML. "Physiologic, pathophysiologic, and pharmacologic regulation of gastric acid secretion". *Current Opinion in Gastroenterology* 33.6 (2017): 430-438.
21. Beales ILP. "Effect of platelet-activating factor on gastric acid secretion and its interaction with interleukin-1 β in *Helicobacter pylori*-infected stomach". *Gut* 42.2 (1998): 227-234.
22. Du H, et al. "Effects of improved sleep and gastric vagotomy on serum inflammatory mediators in peptic ulcer rats". *Neurogastroenterology and Motility* 37.11 (2025): e70136.
23. Percie du Sert N, et al. "The ARRIVE guidelines 2.0: updated guidelines for reporting animal research". *BMJ Open Science* 4.1 (2020): e100115.
24. Takagi K, Okabe S. "The effects of drugs on the production and recovery processes of the stress ulcer". *Japanese Journal of Pharmacology* 19.3 (1969): 418-426.
25. Okabe S, Amagase K. "An overview of acetic acid ulcer models—the history and state of the art of peptic ulcer research". *Biological and Pharmaceutical Bulletin* 28.8 (2005): 1321-1341.

26. Suchecki D., Tufik S. "Sleep rebound in animals deprived of paradoxical sleep by the modified multiple platform technique". *Brain Research* 875.1-2 (2000): 14-22.
27. Koopman FA., *et al.* "Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis". *Proceedings of the National Academy of Sciences of the United States of America* 113.29 (2016): 8284-8289.
28. Bonaz B., *et al.* "Chronic vagus nerve stimulation in Crohn's disease: a 6-month follow-up pilot study". *Neurogastroenterology and Motility* 28.6 (2016): 948-953.

Volume 18 Issue 10 October 2026

©All rights reserved by Hongbin Chen., *et al.*